PCT/AU99/00285

09/673305

Patent Office
Canberra

I, KIM MARSHALL, MANAGER EXAMINATION SUPPORT AND SALES, hereby certify that the annexed is a true copy of the Provisional specification in connection with Application No. PP 2978 for a patent by UNISEARCH LIMITED filed on 16 April 1998.

PRIORITY DOCUMENT
SUBMITTED OR TRANSMITTED IN COMPLIANCE WITH RULE 17.1(a) OR (b)

WITNESS my hand this Twenty-eighth day of April 1999

KIM MARSHALL
MANAGER EXAMINATION SUPPORT AND SALES

AUSTRALIA

Patents Act 1990

UNISEARCH LIMITED

PROVISIONAL SPECIFICATION

Invention Title:

Production of furanones

The invention is described in the following statement:

Technical Field

The present invention relates to the side chain functionalisation of fimbrolides (halogenated 3-alkyl-5-methylene-2(5*H*)-furanones) and their synthetic analogues, that yields fimbrolides substituted with a halogen, an oxygen or a nitrogen functionality in the alkyl chain, especially fimbrolide alcohols, carboxylate and sulfinate esters, ethers, aldehydes, ketones, acids, amides, nitro derivatives, and polymers.

Background Art

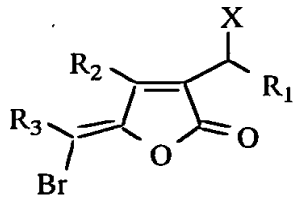
It is known that a variety of fimbrolides possessing antifungal and antimicrobial properties can be isolated from red marine algae *Delisea fimbriata*, *Delisea elegans* and *Delisea pulchra*. The very few reported syntheses of functionalised fimbrolides use (E)-b-bromo-b-lithioacrylate or 3-formyl-6-methylfuran or allenes as starting materials. These syntheses are unnecessarily long, tedious and give very low yields of the fimbrolides. The present inventors have recently reported the preparation of a range of fimbrolides having different sized chain lengths (Manny et al (1997) Tetrahedron 53: 15813-15826).

Prior to the present invention, it had not been appreciated that the side chains of the fimbrolides could be functionalised directly affording a variety of halogen or oxygen functionalised fimbrolides. It has been found that fimbrolides behave like allylic or benzylic compounds in their reactivity and consequently are amenable to free radical functionalisation. The derived halogen compounds can be converted to alcohols or to esters directly from the halogen derivatives or indirectly from the corresponding esters or alcohols respectively. The fimbrolides substituted with an appropriate group in the alkyl chain are capable of yielding polymers through that group, either directly or via copolymerisation with suitable monomers. It is the preparation of these fimbrolide-based halides, alcohols, esters, ethers, ketones, oligomers and polymers that form the major aspect of this invention.

Disclosure of Invention

In a first aspect the present invention consists in a method to form a fimbrolide derivative, the method including reacting a fimbrolide with a halogenating agent and/or an oxygenating agent under appropriate reaction conditions to form compounds with the formula (I), wherein R_1 is any alkyl, alkenyl, aryl or arylalkyl chain or hydrogen; X is a halogen (X = Cl, Br or I) or

oxygen (X = OH, OOH or =O); and R_2 and R_3 are independently or both hydrogen or halogen.



(I)

R_1 = H, alkyl, alkenyl, aryl, arylalkyl
R_2 = H or Br
R_3 = H or Br
X = Cl, Br, I, OH, OOH, $OC(O)R_1$, =O

Preferably the halogenating agent is selected from N-bromosuccinimide, N-chlorosuccinimide, N-iodosuccinimide, bromine, cupric bromide, and phenyltrimethylammonium perbromide. It will be appreciated, however, that other halogenating agents would also be suitable for the present invention.

Preferably the oxygenating agent is selected from lead tetraacetate, Rose Bengal/oxygen gas, hydrogen peroxide/vanadium pentoxide, selenium dioxide, and 3-chloroperoxybenzoic acid. It will be appreciated, however, that other oxygenating agents would also be suitable for the present invention.

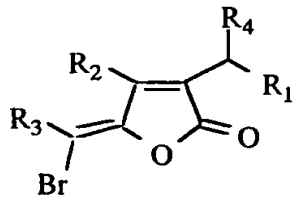
Preferably, the reaction conditions when an halogenating agent is used are carbon tetrachloride or chloroform or dichloromethane/with or without hu/reflux, tetrahydrofuran/room temperature.

Preferably, the reaction conditions when an oxygenating agent is used are acetic acid or acetic acid mixed with a solvent/reflux, pyridine/room temperature, acetone/30°C, dioxane/reflux, and dichloromethane/room temperature.

The present inventors have found that the preferred bromination conditions are N-bromosuccinimide in carbon tetrachloride and hu/reflux. For hu, the present inventors have found that a 250 W lamp is quite suitable.

In a second aspect, the present invention consists in a fimbrolide derivative having formula (I), wherein R_1 is any alkyl, alkenyl, aryl or arylalkyl chain or hydrogen; X is a halogen (X = Cl, Br or I) or oxygen (X = OH, OOH or =O); and R_2 and R_3 are independently or both hydrogen or halogen, with the proviso that the following derivatives are excluded wherein R1=propyl, X=OH, R2=Br, R3=H or Br; and R1=propyl, X=OC(O)CH3, R2=Br, R3=H or Br).

In third aspect, the present invention consists of a method to form a fimbrolide derivative, the method including displacement and/or functionalisation of the halogen or oxygen substituent in the fimbrolide side chain by treating with a nucleophile or an electrophile under appropriate reaction conditions to form compounds with formula (II), wherein R_1 is any alkyl, alkenyl, aryl or arylalkyl chain or hydrogen; R_2 and R_3 are independently or both hydrogen or halogen; and R_4 is halogen, hydroxyl, alkoxy, alkenyloxy, aryloxy, arylalkyloxy, OC(O)R, $OS(O)_2R$, NHC(O)R, or =O.



(II)

R_1 = H, alkyl, alkenyl, aryl, arylalkyl
R_2 = H or Br
R_3 = H or Br
R_4 = F, Cl, Br, I, OH, OR_1, $OC(O)R_1$, $OS(O)R_1$, $NHC(O)R_1$, =O

Preferably the nucleophile is selected from metal halides, water, metal carboxylates, organic alcohols, dimethyl sulfoxide, and acetonitrile/acid catalyst, and silver triflate. It will be appreciated, however, that other nucleophiles would also be suitable for the present invention.

Preferably the electrophile is selected from organic acids, acid chlorides or anhydrides, organic sulfonyl chlorides, and diethylaminosulfur trifluoride. It will be appreciated, however, that other electrophiles would also be suitable for the present invention.

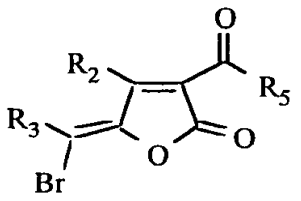
The reaction conditions suitable when using a nucleophile are acetone or dioxane/room temperature or reflux, water/dioxane or acetone or tetrahydrofuran/reflux, metal carboxylates/organic acids/neat or high boiling solvents/reflux, organic alcohols/reflux, dimethyl sulfoxide/room temperature, and acetonitile/acid catalyst or silver triflate/reflux.

The reaction conditions suitable when using a electrophile are organic acids/neat and/or solvent/acid catalyst/reflux, organic acid chlorides or anhydrides/base catalyst/solvent/room temperature, and diethylaminosulfur trifluoride/dichloromethane/low temperature.

In a fourth aspect the present invention consists in a fimbrolide derivative having formula (II), wherein R_1 is any alkyl, alkenyl, aryl or

arylalkyl chain or hydrogen; R_2 and R_3 are independently or both hydrogen or halogen; and R_4 is halogen, hydroxyl, alkoxy, alkenyloxy, aryloxy, arylalkyloxy, OC(O)R, OS(O)R, NHC(O)R, or =O, with the proviso that the following derivatives are excluded wherein R1=propyl, R2=Br, R3=H or Br, R4=OH; and R1=propyl, R2=Br, R3=H or Br, R4=OC(O)CH3).

In a fifth aspect, the present invention consists a method to form a fimbrolide derivative (aldehydes, acids and ketones), the method including reacting a hydroxyl substituent in the fimbrolide side chain with an oxidation agent under appropriate reaction conditions to form a compound with formula (III) wherein R_1 is any alkyl, alkenyl, aryl or arylalkyl chain or hydrogen; R_2 and R_3 are independently or both hydrogen or halogen; and R_5 is OH or the same as R_1.



R_2 = H or Br
R_3 = H or Br
R_5 = OH or R_1
R_1 = H, alkyl, alkenyl, aryl, arylal

(III)

Preferably, the oxidation agents are acid dichromate reagents in any form either free or polymer supported (e.g. Jones reagent, pyridinium chlorochromate, pyridinium dichromate *etc*), manganese dioxide, potassium permanganate, selenium dioxide, ceric ammonium nitrate, ruthenium tetraoxide, and hot nitric acid. It will be appreciated, however, that other oxidation agents may also be used for the present invention.

The reaction conditions preferably use Jones reagent/with or without phase transfer catalysts/acetone/room temperature, toluene/reflux, potassium permanganate/buffered solution/room temperature, dioxane/reflux, ceric ammonium nitrate/ aqueous acetic acid/steam bath, carbon tetrachloride/reflux, and acetic acid/steam bath. It will be appreciated, however, that other reaction conditions may also be used for the present invention.

The present inventors have found that the use of Jones reagent in acetone/room temperature is particularly suitable.

In a sixth aspect, the present invention consists in a fimbrolide derivative having the formula (III) wherein R_1 is any alkyl, alkenyl, aryl or arylalkyl chain or hydrogen; R_2 and R_3 are independently or both hydrogen or halogen; and R_5 is OH or the same as R_1.

In a seventh aspect, the present invention consists in incorporation of fimbrolides according to the present invention either in surface coatings or polymers through the newly introduced functionality on the alkyl chain or the alkyl chain itself via direct polymerisation or copolymerisation with suitable monomers.

In an eighth aspect, the present invention consists in a fimbrolide derivative produced by the method according to the first, third, or fifth aspects of the present invention.

In a ninth aspect, the present invention consists in the use of a fimbrolide derivative according to the present invention. The present inventors have found that many of the fimbrolide derivatives having the formula (I), (II), and (III) have antimicrobial and/or antifouling properties. Accordingly, the fimbrolide derivatives are suitable for use as antimicrobial and/or antifouling agents.

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

In order that the present invention may be more clearly understood, preferred forms will be described with reference to the following examples and accompanying drawings.

Brief Description of Drawings

Figure 1 shows the structure of *Delisea pulchra* furanones and synthetic analogues and derivatives tested in the barnacle settlement assay.

Figure 2 shows the effect of furanones 2, 281, 2223, 2425, 26, 27 and 28 on the settlement of barnacle cyprid larvae as measured by settlement expressed as a percent of the control.

Figure 3 shows growth curves of *Staphylococcus aureus* against different furanones.

Figure 4 shows growth curves of *Staphylococcus aureus* against compounds 33/34 and 45.

Modes for Carrying Out the Invention

EXPERIMENTAL DETAILS

Fimbrolide Production

General. Melting points are uncorrected. Microanalyses were performed by Dr H.P. Pham of The University of New South Wales Microanalytical Laboratory. ^1H NMR spectra were obtained in $CDCl_3$ on a Bruker AC300F (300 MHz) or a Bruker DMX500 (500 MHz) spectrometer. ^{13}C NMR were obtained in the same solvent on a Bruker AC300F (75.5 MHz) or a Bruker DMX500 (125.8 MHz) spectrometer. Chemical shifts were measured on the δ scale internally referenced to the solvent peaks: $CDCl_3$ (δ 7.26, δ 77.04). Ultraviolet spectra were measured on an Hitachi U-3200 spectrophotometer and refer to solutions in absolute MeOH. Infrared spectra were recorded on a Perkin-Elmer 298 or a Perkin-Elmer 580B spectrophotometer and refer to paraffin mulls. The electron impact mass spectra were recorded on an VG Quattro mass spectrometer at 70eV ionisation voltage and 200°C ion source temperature. FAB spectra were recorded on an AutoSpecQ mass spectrometer. Column chromatography was carried out using Merck silica gel 60H (Art. 7736), whilst preparative thin layer chromatography was performed on 2 mm plates using Merck silica gel $60GF_{254}$ (Art. 7730).

RESULTS

Fimbrolide Production

Examples of a number of fimbrolides produced are provided below.

4-Bromo-5-(bromomethylene)- and 5-(dibromomethylene)-3-(1-bromoethyl)-2(5H)-furanone

N-bromosuccinimide (17.3 g, 0.097 mol) was added to a solution of 4-bromo-5-(bromomethylene)- and/or 5-(dibromomethylene)-3-ethyl-2(3H)-furanone (22.6 g, 0.08 mol) in carbon tetrachloride (500 ml) containing benzoyl peroxide (0.25 g). The mixture was irradiated with a 250 W lamp and refluxed in an oil bath for 18h. After cooling the mixture to room temperature it was filtered and the precipitate washed with carbon tetrachloride (50 ml). The filtrate was evaporated under reduced pressure and the crude product was purified by silica gel chromatography using dichloromethane / light petroleum (2 : 3) as the eluent to yield the bromo compounds (22.0 g, 76%).

4-Bromo-5-(bromomethylene)-3-(1-bromoethyl)-2(5H)-furanone

A pale yellow solid, m.p. 79°C. n_{max} 2850, 1750, 1630, 1580, 1440, 1360, 1270, 1180, 1065, 1000, 970, 940, 1080, 755 cm^{-1}. l_{max} 306 nm (e 10826). ^1H n.m.r. d ($CDCl_3$) 2.06, d, J 7.2 Hz, $(H2')_3$; 5.00, q, J 7.2 Hz, H1'; 6.45, s, 5-CHBr. ^{13}C n.m.r. d ($CDCl_3$): 22.3, C2'; 35.7, C1'; 94.3, 5-CHBr; 130.5, C4; 133.7, C; 149.5, C5; 165.8, C2. Mass spectrum: m/z 364 (M ($^{81}Br_3$), 2%); 362 (M ($^{81}Br_2$, ^{79}Br), 8); 360 (M (^{81}Br $^{79}Br_2$), 8); 358 (M ($^{79}Br_3$), 2); 283 (85); 281 (100); 279 (85); 202 (12); 200 (12); 173 (18); 158 (35); 156 (35); 145 (38); 143 (42); 133 (28); 121 (26).

5-(Dibromomethylene)-3-(1-bromoethyl)-2(5H)-furanone

A white solid m.p. 119°C. n_{max} 2900, 1720, 1590, 1450, 1370, 1250, 1170, 1080, 1060, 1000, 960, 840, 770, 720 cm^{-1}. l_{max} 319 nm (e 12225). ^1H n.m.r. d ($CDCl_3$): 1.99, t, J 7.2Hz, $(H2')_3$; 4.87, q, J 7.2 Hz, H1'; 7.56, s, H4. ^{13}C n.m.r. d ($CDCl_3$): 23.9, C2'; 36.0, C1'; 82.8, (5-CBr_2); 134.7, C4; 138.2, C3; 149., C5; 165.5, C2. Mass spectrum: m/z 364 (M ($^{81}Br_3$), 9%); 362 (M ($^{81}Br_2$, ^{79}Br), 18); 360 (M (^{81}Br $^{79}Br_2$), 18); 358 (M ($^{79}Br_3$), 9); 283 (78); 281 (100); 279 (78); 227 (8); 225 (12); 223 (8); 202 (22); 200 (32); 174 (18); 172 (44); 146 (42); 145 (50); 144 (50); 143 (60).

5-(Dibromomethylene)-3-(1-hydroxyethyl)-2(5H)-furanone

A solution of 5-(dibromomethylene)-3-(1-bromoethyl)-2(5H)-furanone (18.0 g, 0.05 mol) in a mixture of dioxane (200 ml) and water (105 ml) was refluxed overnight. After cooling the mixture to room temperature, it was diluted with water (300 ml) and extracted with ether (3 x 200 ml). The combined ether extracts were washed with water, dried and evaporated. The crude product was purified by silica gel chromatography using dichloromethane / light petroleum (1 : 1) as an eluent to yield the hydroxyethyl furanone (4.80 g, 31%) as a white solid. m.p. 100°C. n_{max} 3300, 2870, 1750, 1595, 1440, 1370, 1250, 1170, 1030, 985, 955, 835, 770, 720 cm^{-1}. l_{max} 311 nm (e 5832). ^1H n.m.r. d ($CDCl_3$) 1.50, d, J 7.2 Hz, $(H2')_3$; 4.72, m, 1H, H1'; 7.49, bs, H4. ^{13}C n.m.r. d ($CDCl_3$): 21.8, C2'; 63.4, C1'; 81.3, 5-CHBr; 133.7, C4; 140.3, C3; 149.5, C5; 167.3, C2. Mass spectrum: m/z 300, (M+1 ($^{81}Br_2$), 18%); 298 (M+1 (^{81}Br, ^{79}Br), 36); 296, (M+1 ($^{79}Br_2$), 18); 285 (22); 283 (41); 281 (28); 257 (78); 255 (100); 253 (78); 219 (15); 217 (15); 201 (22); 200 (34); 199 (36); 174 (24); 172 (38); 170 (18); 147 (21); 145 (28); 119 (38); 117 (38).

4-Bromo-5-(bromomethylene)- and 5-(dibromomethylene)- 3-(1-acetoxybutyl)-2(5H)-furanone

A solution of 4-bromo-5-(bromomethylene)- and/or 5-(dibromomethylene)- 3-(1-bromobutyl)-2(5H)-furanone (3.00 g, 7.7 mmol) in glacial acetic acid (160 ml) containing sodium acetate (1.20 g, 15 mmol) was refluxed for 18h. The mixture was concentrated to approximately 20 ml and neutralised with excess saturated sodium carbonate solution. The residual oil was extracted with ether (3 x 100 ml), washed with brine, dried over sodium sulfate and evaporated. The crude product was chromatographed on silica gel using dichloromethane / light petroleum (1 : 1) as eluent to yield the acetoxybutylfuranones (0.96 g, 34%).

4-Bromo-5-(bromomethylene)-3-(1-acetoxybutyl)-2(5H)-furanone

A pale yellow oil n_{max} 2940, 1775, 1740, 1640, 1600, 1450, 1420, 1370, 1220, 1100, 1020, 985, 760, 730 cm^{-1}. l_{max} 295 nm (e 6265). ^1H n.m.r. d ($CDCl_3$) 0.93, t, J 7.2 Hz, $(H4')_3$; 1.35, m, $(H3')_2$; 1.84, m, $(H2')_2$; 2.07, s, $COCH_3$; 5.50, bt, J 7.2 Hz, H1'; 6.37, s, 5-CHBr. ^{13}C n.m.r. d ($CDCl_3$): 13.5, C4'; 18.5, $COCH_3$; 20.6, C3'; 33.7, C2'; 68.2, C1'; 93.5, 5-CHBr; 130.2, C4; 131.4, C3; 149.7, C5; 164.2, C2; 170.2, CO. Mass spectrum: m/z 370, (M ($^{81}Br_2$), <5%); 368 (M (^{81}Br, ^{79}Br), <5); 366, (M ($^{79}Br_2$), <5); 327 (18); 325 (26); 323 (18); 289 (22); 287 (22); 285 (14); 283 (28); 281 (14); 247 (12); 245 (12); 229 (14); 227 (14); 149 (28).

5-(dibromomethylene)-3-(1-acetoxybutyl)-2(5H)-furanone

A pale yellow solid mp 76°C. n_{max} 2880, 1760, 1735, 1445, 1370, 1225, 1170, 1100, 1030, 950, 840, 765, 7320 cm^{-1}. l_{max} 314 nm (e 8900). ^1H n.m.r. d ($CDCl_3$) 0.94, t, J 7.2 Hz, $(H4')_3$; 1.36, m, $(H3')_2$; 1.84, m, $(H2')_2$; 2.12, s, $COCH_3$; 5.59, bt, J 6.2 Hz, H1'; 7.39, bs, H4. ^{13}C n.m.r. d ($CDCl_3$): 13.6, C4'; 18.3, $COCH_3$; 20.9, C3'; 34.8, C2'; 68.3, C1'; 81.6, 5-CHBr; 135.0, C4; 136.1, C3; 149.3, C5; 166.1, C2; 169.9, CO. Mass spectrum: m/z 370, (M ($^{81}Br_2$), 28%); 368 (M (^{81}Br, ^{79}Br), 54); 366, (M ($^{79}Br_2$), 28); 328 (20); 327 (18); 326 (36); 325 (28); 324 (20); 323 (18); 289 (16); 287 (16); 247 (16); 245 (16); 229 (12); 227 (12); 198 (10).

5-(Dibromomethylene)-3-(1-acetoxyethyl)-2(5H)-furanone

The procedure described for 4-bromo-5-(bromomethylene)-3-(1-acetoxybutyl)-2(5H)-furanone was used to treat 5-(dibromomethylene)-3-(1-bromoethyl)-2(5H)-furanone (2.80 g, 7.7 mmol) with sodium acetate (1.20 g, 15 mmol) in glacial acetic acid (160 ml) to give after chromatography the

acetoxyethyl furanone as a white solid (0.88 g, 34%) m.p. 124°C. ν_{max} 2880, 1750, 1610, 1445, 1365, 1230, 1170, 1080, 1030, 990, 960, 930, 835, 760, 715 cm^{-1}. λ_{max} 313 nm (e 31296). ^1H n.m.r. d ($CDCl_3$) 1.53, d, J 6.2 Hz, $(H2')_3$; 2.13, s, $COCH_3$; 5.66, m, 1H, H1'; 7.43, bs, H4. ^{13}C n.m.r. d ($CDCl_3$): 18.9, CH_3; 20.9, C2'; 53.4, C1'; 81.7, 5-CHBr; 134.6, C4; 136.7, C3; 149.2, C5; 166.0, C2; 169.6, CO. Mass spectrum: m/z 342, (M ($^{81}Br_2$), <5%); 340 (M (^{81}Br, ^{79}Br), 6); 338, (M ($^{79}Br_2$), <5); 300 (30); 299 (26); 298 (62); 297 (44); 296 (32); 295 (22); 281 (22); 279 (18);261 (34); 259 (37); 219 (68); 217 (70); 201 (32); 200 (31); 199 (34); 174 (20); 172 (30); 170 (14); 157 (22); 145 (28); 143 (24).

4-Bromo-5-(bromomethylene)-3-(1-butyroyloxybutyl))-2(5H)-furanone

4-Bromo-5-(bromomethylene)-3-(1-hydroxybutyl))-2(5H)-furanone (4.75 g, 0.015 mol) and butyroyl chloride (7.8 ml, 0.075 mol) were refluxed together for 7h then cooled and poured into water (50 ml) and extracted with ether (3 x 30 ml). The combined ether extracts were washed sequentially with saturated sodium bicarbonate (2 x 50 ml) and brine (50 ml), dried over sodium sulfate, and evaporated. The crude product was purified by silica gel chromatography using ether / light petroleum (1 : 9) as the eluent to yield the butyryloxybutyl furanone as a pale yellow oil (3.60 g, 60%). ν_{max} 2950, 1780, 1730, 1635, 1600, 1450, 1380, 1280, 1240, 1165, 1060, 980, 840, 770 cm^{-1}. λ_{max} 289 nm (e 14900). ^1H n.m.r. d ($CDCl_3$) 0.91, t, J 7.4 Hz, $OCOCH_2CH_2C\underline{H}_3$; 0.93, t, J 7.2 Hz, $(H4')_3$; 1.35, m, $(H3')_2$; 1.66, q, J 7.4 Hz, $OCOCH_2C\underline{H}_2CH_3$; 1.80-1.95, m, $(H2')_2$; 2.32, t, J 7.4 Hz, $OCOC\underline{H}_2CH_2CH_3$; 5.50, dd, J 6.4 Hz 8.0 Hz, H1'; 6.36, s, 5-CHBr. ^{13}C n.m.r. d ($CDCl_3$): 13.4, $OCOCH_2CH_2\underline{C}H_3$; 13.5, C4'; 18.2, $OCOCH_2\underline{C}H_2CH_3$; 18.4, C3'; 33.5, C2'; 35.7, $OCO\underline{C}H_2CH_2CH_3$; 68.0, C1'; 93.2, 5-CHBr; 130.6, C4; 132.4, C3; 149.6, C5; 165.9, C2; 172.7, CO. Mass spectrum: m/z 399, (M+1 ($^{81}Br_2$), <5%); 397 (M+1 (^{81}Br, ^{79}Br), <5); 395, (M+1 ($^{79}Br_2$), <5); 327 (18); 325 (28); 323 (18); 317 (26); 315 (26); 311 (8); 309 (16); 307 (8); 283 (16); 281 (34); 279 (16); 267 (42); 265 (40); 247 (16); 245 (16); 223 (56); 221 (44).

4-Bromo-5-(bromomethylene)-3-(1-acryloyloxybutyl))-2(5H)-furanone

The procedure described for 4-bromo-5-(bromomethylene)-3-(1-butyroyloxybutyl))-2(5H)-furanone was used to treat 4-bromo-5-(bromomethylene)-3-(1-hydroxybutyl))-2(5H)-furanone (4.75 g, 0.015 mol) with acryloyl chloride (6.0 ml, 0.073 mol). The crude product was purified by silica gel chromatography using ether / light petroleum (1 : 9) as the eluent to yield the acryloyloxybutyl furanone as a pale yellow oil (3.60 g, 60%).

n_{max} 3060, 2940, 2850, 1770, 1710, 1620, 1590, 1430, 1390, 1385, 1280, 1250, 1160, 1095, 1030, 970, 835, 795, 760, 700 cm^{-1}. l_{max} 293 nm (e 18170). ^1H n.m.r. d ($CDCl_3$) 0.91, t, J 7.4 Hz, ester CH_3; 0.97, t, J 7.4 Hz, $(H4')_3$; 1.38, m, $(H3')_2$; 1.84-2.04, m, $(H2')_2$; 5.63, dd, J 6.7 Hz 8.2 Hz, H1'; 5.88, d, J 10.7 Hz, CH=CH2; 6.14, dd, J 10.7 Hz 16.3 Hz, $CH=CH_2$; 6.39, s, 5-CHBr; 6.46, d, J 16.3 Hz, $CH=CH_2$. ^{13}C n.m.r. d ($CDCl_3$): 13.5, C4'; 18.5, C3'; 33.7, C2'; 68.2, C1'; 93.5, 5-CHBr; 127.5, $CH=CH_2$; 130.4, C4; 131.5, $CH=CH_2$; 132.1, C3; 149.8, C5; 163.7, C2; 165.2, CO. Mass spectrum: m/z 382, (M ($^{81}Br_2$), <5%); 380 (M (^{81}Br, ^{79}Br), <5); 378, (M ($^{79}Br_2$), <5); 327 (14); 325 (28); 323 (14); 301 (16); 299 (16); 283 (8); 281 (12); 279 (8); 269 (12); 267 (24); 265 (12); 229 (18); 227 (24); 225 (18); 223 (20); 203 (34); 201 (46); 175 (32); 173 (48); 147 (38); 145 (46); 143 (48).

Fimbrolide Biological Activity

MATERIALS AND METHODS

Inhibition of Cyprid Settlement

The effects of synthetic furanones on the settlement of barnacle larvae were tested using cyprids of the cosmopolitan fouling barnacle *Balanus amphitrite* Darwin. The naturally occurring furanone 2, and the synthetically prepared compounds 281 (a 1:1:1 mixture of synthesised 2 & 8 & 1), 2223 (a 1:1 mixture of synthesised 22 & 23), 2425 (a 1:1 mixture of synthesised 24 & 25), 26, 27, and 28 (Figure 1) were compared for their efficacy in deterring barnacle cyprid settlement. Compounds were dissolved in ethanol (99.7% + purity) at a concentration of 180 $\mu g.ml^{-1}$ to 1.8 $\mu g.ml^{-1}$. A 0.5 ml aliquot of each compound to be tested was added to treatment petri dishes (surface area 9 cm^2), and 0.5 ml of ethanol only was added to ethanol control dishes. Dishes were dried on a shaker resulting in a coating of extract on treatment dishes with a concentration range of 10 $\mu g.cm^{-2}$ to 100 $ng.cm^{-2}$ for each compound.

Cypris larvae were obtained from laboratory cultures of adult brood stock of *Balanus amphitrite*. Nauplii of *B. amphitrite* were collected and reared on *Skeletonema costatum* until reaching cyprid stage. Cypris larvae were filtered and maintained in filtered seawater at 5°C for five days prior to use in settlement assays (Rittschof *et al.*, 1992).

Settlement tests were conducted by adding 25-35 cyprids to either treatment dishes, ethanol control dishes, or untreated dishes, each containing 4 ml of sterilised filtered seawater (0.22 μm). All the treatments and controls

were tested in triplicate. Test dishes were incubated for 24 h at 28°C in a 15:9 h light-dark cycle (Rittschof *et al.*, 1992). After 24 h, the test was terminated by the addition of three drops of 40% formaldehyde, and non-settled larvae filtered from the dish. The percent settlement of cyprids was then determined by counting settled and non-settled larvae.

Statistical Analyses

The data from the bioassays were analysed by analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Data were analysed as percentages after arcsin Āp transformations.

RESULTS

Inhibition of Cyprid Settlement

The settlement of *Balanus amphitrite* Cypris larvae was significantly inhibited by the compounds tested (Figure 2; two-factor ANOVA [metabolite x concentration] followed by Tukey's test). All treatments completely inhibited settlement at the highest concentration (10 $\mu g.cm^{-2}$). Ethanol controls were used in the analysis as ethanol had no significant effect on settlement (single factor ANOVA , P=0.17). The synthetic furanone 2223 (Figure 1) was the most active metabolites (Figure 2). At a concentration of 1 $\mu g.cm^{-2}$ 2223 completely inhibited settlement and inhibited settlement by 80% compared to the control at 500 $ng.cm^{-2}$. The next most inhibitory compound was the furanone 28 (Figure 1) which inhibited settlement completely at 5 $\mu g.cm^{-2}$ and inhibited settlement by 90% at 1 $\mu g.cm^{-2}$. A group of furanones, 2425, 26 and 27 completely inhibited settlement at 5 $\mu g.cm^{-2}$ but had no effect at 1 $\mu g.cm^{-2}$. The furanone 2 and the synthetic analogue 281, a 1:1:1 mixture of 2, 8 and 1 (Figure 1) were the least effective compounds completely inhibiting settlement at 10 $\mu g.cm^{-2}$.

Inhibition of *Staphylococcus aureus*

Staphylococcus aureus is a facultatively anaerobic, nonmotile, gram-positive coccus and is normally associated with the skin, skin glands, and mucous membranes of humans. *S. aureus* is the most important human staphylococcal pathogen and causes, for example, boils, abscesses and wound infections.

A screening experiment of the different furanones against the growth of *S. aureus* was performed in a BioRad 3550 Microplate reader. The growth was measured as absorbance at 610 nm up to 9 h. A complex growth media, Nutrient Broth, was used and the cells were grown at 37°C. Both natural

furanones (compounds 2, 3 and 4) and synthesised furanones (compounds 33/34 and 45) were used in the experiment at the concentration 10 μg/ml.

The results showed that the synthesised furanones (33/34 and 45) inhibited growth of *S. aureus* more effectively than the natural furanones (Figure 3). The growth of the cells inoculated with 33/34 and 45 was completely inhibited for 9 h compared to 2 h for those inoculated with the natural compounds. All furanones, however, inhibited the growth of *S. aureus* compared to the control.

Further experiments were performed with the synthesised furanones 45 and 33/34 at the concentrations 10 μg/ml and 5 μg/ml. The cells were grown in side arm flasks in NB media at 37^{0}C. The growth of the cells were measured at 610 nm for up to 48 h.

The results showed that compound 33/34 was more effective at inhibiting growth of *S. aureus* compared to compound 45 (Figure 4), however, both compounds at both concentrations inhibited the growth completely for 9 h. Growth of the cells occurred after 9h with compound 45 at the concentration 5μg/ml and after 15 h at the concentration 10 μg/ml. Compound 33/34 at 5μg/ml inhibited the growth for 15 h and at the concentration 10 μg/ml the growth of *S aureus* was completely inhibited for 34 h.

DISCUSSION

The derivatisation of naturally occurring furanones resulted in an increase in the deterrence of barnacle settlement. For example, manipulation of the length of the acyl side chain and the functionality on the 1' position of the acyl side chain of the furanone resulted in a significant increase in activity. This is clearly demonstrated in a comparison of the activity of furanones 2 and 2425. In 2425 a bromine has been added in the 1' position of acyl chain resulting in a five fold increase in activity in the settlement bioassay (Figure 2). All of the synthesised furanones are either novel compounds not being previously reported in the literature or are racemic mixtures of a naturally occurring furanone. The racemic analogues of the naturally occurring compounds have the same activity as the naturally occurring optically pure form. Therefore, the synthetic furanones, both analogues of naturally occurring compounds and novel compounds, have activity comparable to or better than the compounds from which their structure was derived, e.g. furanone 2 vs 2425.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

Dated this 16th day of April 1998

UNISEARCH LIMITED
Patent Attorneys for the Applicant:

F B RICE & CO

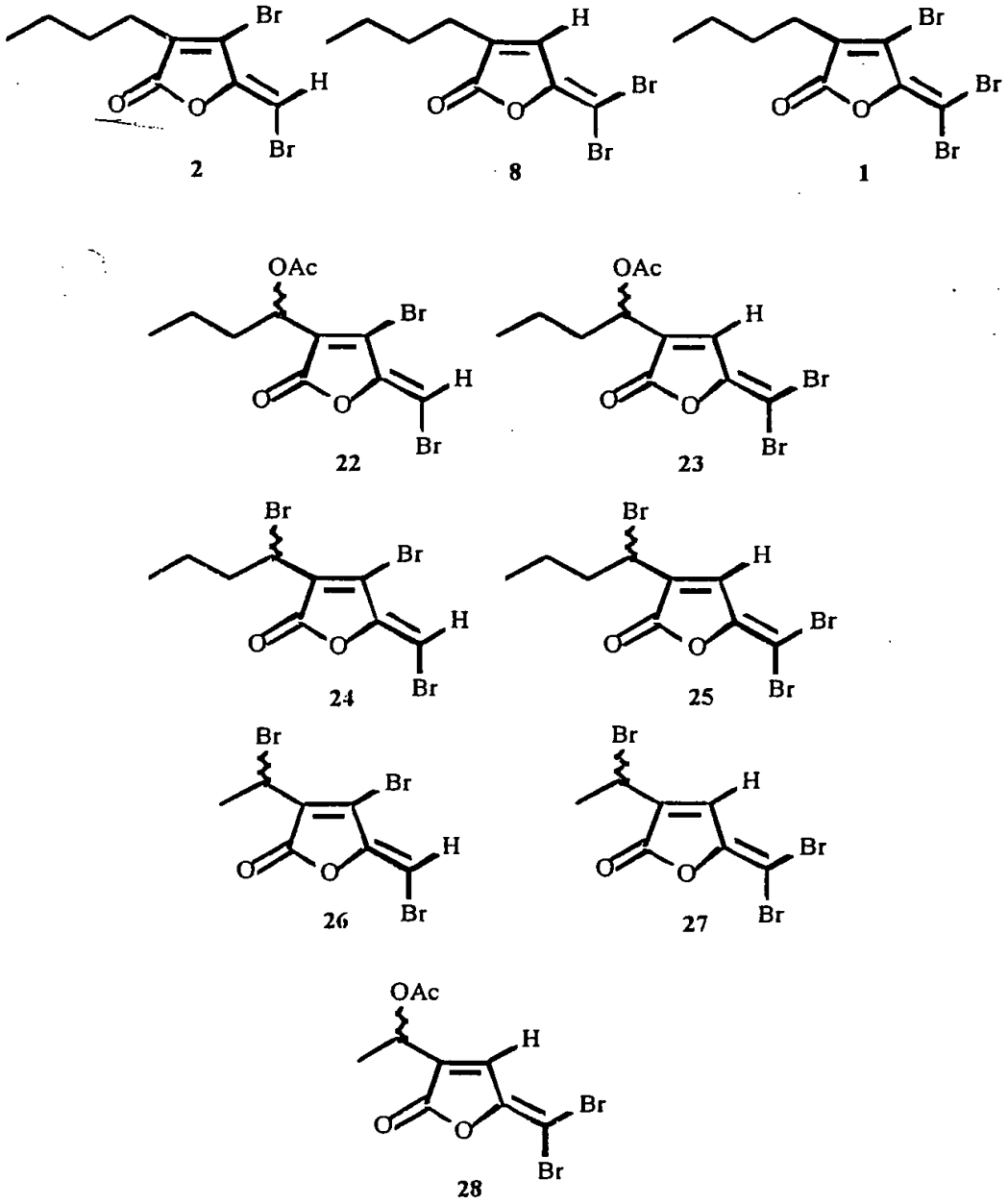

Br
H
Br
O
O
2
H
Br
Br
O
O
8
Br
Br
Br
O
O
1
OAc
Br
H
Br
O
O
22
OAc
H
Br
Br
O
O
23
Br
Br
H
Br
O
O
24
Br
H
Br
Br
O
O
25
Br
Br
H
Br
O
O
26
Br
H
Br
Br
O
O
27
OAc
H
Br
Br
O
O
28

Figure 1

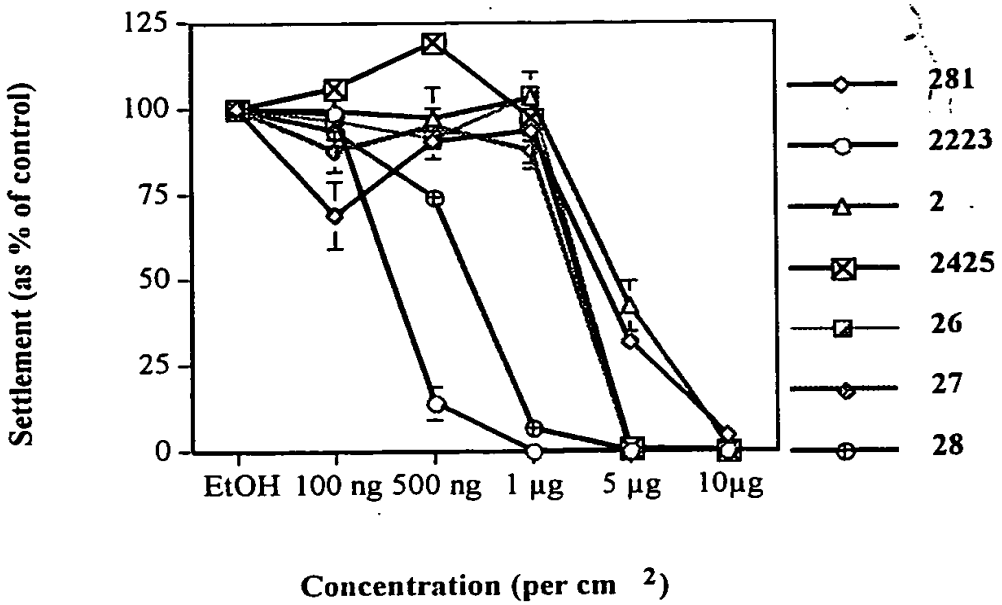

Settlement (as % of control)
125
100
75
50
25
0
EtOH
100 ng
500 ng
1 µg
5 µg
10µg
Concentration (per cm 2)
281
2223
2
2425
26
27
28

Figure 2

Growth curves of *Staphylococcus aureus* against different furanones

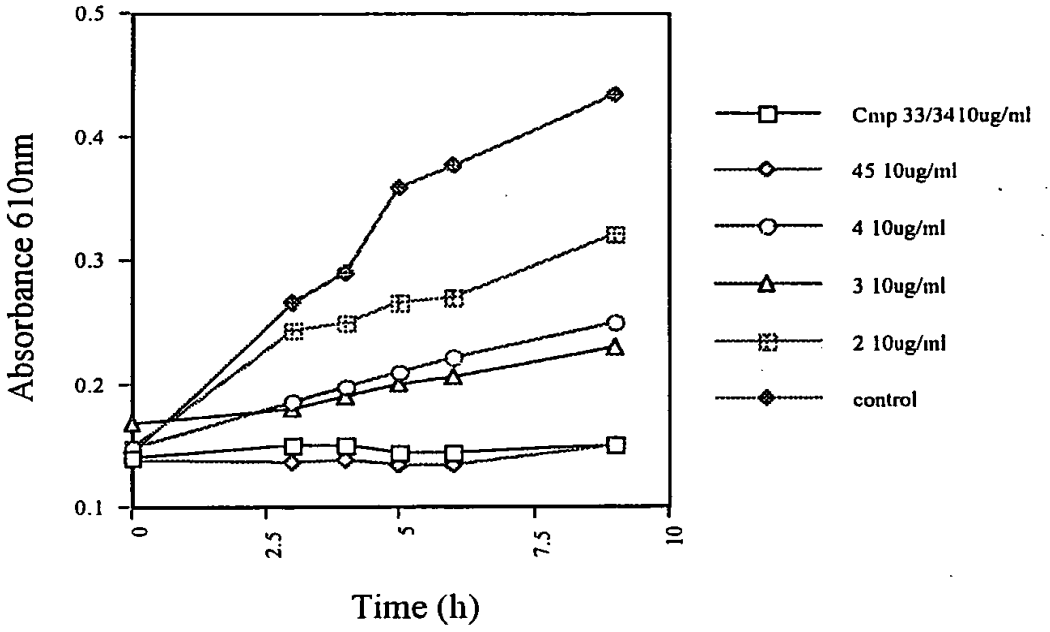


Figure 3

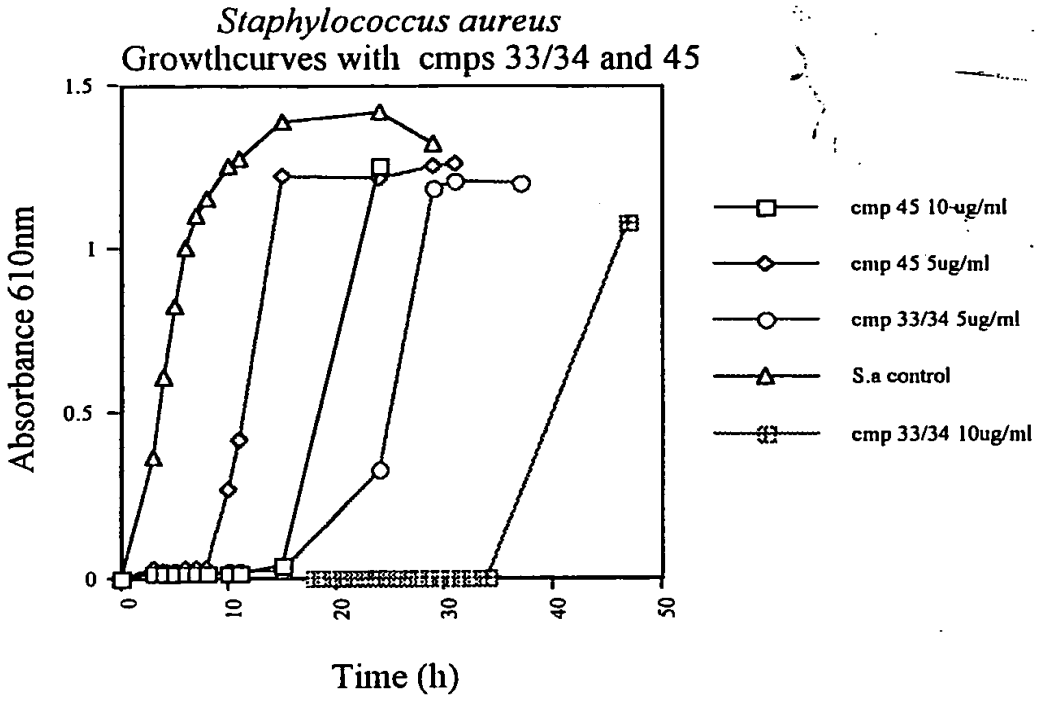

Staphylococcus aureus
Growthcurves with cmps 33/34 and 45
Absorbance 610nm
1.5
1
0.5
0
0
10
20
30
40
50
Time (h)
cmp 45 10-ug/ml
cmp 45 5ug/ml
cmp 33/34 5ug/ml
S.a control
cmp 33/34 10ug/ml

Figure 4